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# Optimal Parameters for Laser Tissue Soldering: II. Premixed Versus Separate Dye-Solder Techniques

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***Background and Objective:*** **Laser tissue soldering by using an indocyanine green (ICG)-doped protein solder applied topically to the tissue surface and denatured with a diode laser was investigated in Part I of this study. The depth of light absorption was predominantly determined by the concentration of the ICG dye added to the solder. This study builds on that work with an in vitro investigation of the effects of limiting the zone of heat generation to the solder-tissue interface to determine whether more stable solder-tissue fusion can be achieved.**
***Study Design/Materials and Methods:*** **An alternative laser tissue soldering technique was investigated, which increased light absorption at the vital solder-tissue interface. A thin layer of ICG dye was smeared over the surface to be treated, the protein solder was then placed directly on top of the dye, and the solder was denatured with an 808-nm diode laser. Because laser light at ~800 nm is absorbed primarily by the ICG dye, this thin layer of ICG solution restricted the heat source to the space between the solder and the tissue surfaces. A tensile strength analysis was conducted to compare the separate dye-solder technique with conventional techniques of laser tissue soldering for which a premixed dye-solder is applied directly to the tissue surface. The effect of hydration on bond stability of repairs formed by using both techniques was also investigated using tensile strength and scanning electron microscopy analysis.**
***Results:*** **Equivalent results in terms of tensile strength were obtained for the premixed dye-solder technique using protein solders containing 0.25 mg/ml ICG (liquid solder, 220 ± 35 N/cm$^2$; solid solder, 602 ± 32 N/cm$^2$) and for the separate dye-solder technique (liquid solder, 228 ± 41 N/cm$^2$; solid solder, 578 ± 29 N/cm$^2$). The tensile strength of native bovine thoracic aorta was 596 ± 31 N/cm$^2$. Repairs created by using the separate dye-solder technique were more stable during hydration than their premixed dye-solder counterparts. The conventional premixed**

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**dye-solder was simpler and approximately twice as fast to apply. The separate dye-solder technique, however, increased the shelf-life of the solder, because the dye was mixed at the time of the experiment, thus conserving its spectral absorbency properties.**
***Conclusion:*** **Two laser-assisted tissue soldering techniques have been evaluated for repairing aorta incisions in vitro. The advantages and disadvantages of each of these techniques are discussed. Lasers Surg. Med. 26:346–356, 2000** 

**Key words: diode laser; indocyanine green dye; protein solder; tensile strength; tissue repair; scanning electron microscopy**

## INTRODUCTION

Laser tissue welding is increasing in popularity as a new and attractive technique for tissue repair over conventional suture techniques. However, clinical use of this technique has been hindered by unreliable fusion strength [1,2], excessive thermal damage of tissue caused by direct laser heating [3–5], technical difficulties with tissue alignment [6], the ambiguity of the end point for the procedure [6], and poor reproducibility [7]. Laser soldering by using protein-based biological glues and other compounds can provide greater bond strength and less collateral damage with a greater tolerance of parameter variations. Useful solders include blood [8,9], cryoprecipitate [2,10], and albumin [11–13]. Suitable lasers deliver wavelengths that are highly absorbed either by water or the tissue's natural chromophores [14, 15]. For example, argon lasers (488 and 514 nm) and KTP lasers (532 nm) are used with hemoglobin, and Nd:YAG (1.064 and 1.320 nm), Ho:YAG (2.06 μm), Th:YAG (2.14 μm), Er:YAG (2.94 μm), and $CO_2$ (10.6 μm) lasers are used with water. Endogenous and exogenous materials such as indocyanine green (ICG) are often added to solders to enhance light absorption [11,16–20]. ICG dye has a maximum absorption coefficient at 805 nm of $2 \times 10^5$ $mg^{-1}$ $cm^{-1}$. The dye binds preferentially with serum protein [21], and when used with a diode laser (790 to 830 nm), it ensures that heat is efficiently transferred to denature the protein solder. Independent of the choice of chromophore, more energy is generally absorbed near the upper portion of the solder, closer to the laser source. A temperature gradient is established over the depth of the solder. Depending on the temperature gradient and the laser exposure, the upper portion of the solder can become overcoagulated while the most critical region, the solder/tissue interface, does not get fully coagulated. Such undercoagulated solder has been shown to create unstable bonds with tissue [22].

In Part I of this study, we investigated optimal parameters for maximum tensile strength by using ICG-doped liquid and solid protein solders in which the depth of light absorption is simply determined by the dye concentration [23]. In Part II, we build on previous work by limiting the absorption of laser light to the region of the solder-tissue interface to determine whether more stable solder-tissue coagulation can be achieved. Rather than mixing the absorbing dye in the protein solder, a thin layer of dye is smeared over the surface to be treated and the protein solder is then placed directly on top of the dye. Because the laser light is primarily absorbed by the ICG dye, this thin layer of ICG solution forms the heat source to the protein solder and to the tissue surface. The results are compared with specimens repaired using the premixed dye-solder technique.

A comparative study of the conventional premixed dye-solder vs. the separate dye-solder technique for laser-solder repair of tissue was conducted with bovine aorta tissue. The investigation was divided into two parts, during which a total of 1,460 tissue repairs were performed and tested. Tensile strength analysis was performed to test the integrity of the resultant repairs immediately after the laser procedure for a range of laser irradiances and exposure times. The effect of hydration on bond stability was also investigated, through tensile strength and scanning electron microscopy (SEM) analysis. Both liquid (25% bovine serum albumin [BSA]) and solid (60% BSA) protein solders, containing either 0.25 mg/ml ICG (penetration depth of 85 μm) or no ICG, were used in this investigation.

## MATERIALS AND METHODS

### Protein Solder Preparation

Liquid protein solder solution was prepared from 25% (w/v) BSA (Sigma Chemical Co.) mixed in deionized water. The protein solder was stored

in a light-proof glass vial in a refrigerator until required. Solution remaining after 10 days was discarded.

Solid protein solder strips were prepared from 60% (w/v) BSA mixed in deionized water. The mixture was pressed to a thickness of 0.15 ± 0.01 mm and then cut into rectangular strips having nominal dimensions of 3 × 1 mm and allowed to dry. The solid protein solder strips were stored in a light-proof container between two inert metal plates in a refrigerator until required. Strips not used within 10 days were discarded.

For the premixed dye-solder technique, ICG dye (Becton Dickinson) with a concentration of 0.25 mg/ml was added to enhance light absorption. This concentration of dye produced the strongest repairs in Part I of this study [23]. For the separate dye-solder technique, a separate ICG solution (concentration of 10 mg/ml) was made up with deionized water. Before use, the protein solders and ICG dye solution were allowed to reach room temperature.

### Laser System

A GaA1As semiconductor laser diode with a nominal output power of 1.5 W and wavelength of 808 nm (Endodiode 10000, Alcon Surgical, Inc.) was used to denature the protein solder. For the tensile strength analysis, the laser radiation was delivered through a 400-μm-core silica fiber ($1/e^2$ spot size of ~1 mm at the solder surface) as described in Part I of this study. Diode powers of 50, 100, 150, 200, and 250 mW, measured with a power meter (Coherent Fieldmaster), were delivered to the protein solder surface, resulting in irradiances of 6.4, 12.7, 19.1, 25.5, and 31.8 W/cm$^2$, respectively. Exposure times of 40, 60, 80, 100, and 200 seconds were used for the liquid protein solder, and exposure times of 20, 30, 40, 50, and 100 seconds were used for the solid protein solder.

For the hydration study, the laser radiation was focused through a series of optics onto the specimen, which was held at a fixed distance from the optics ($1/e^2$ spot size of ~1.5 mm at the solder surface). Losses through the optics reduced the laser power by 71%. Diode powers of 300, 400, 500, and 750 mW, measured with a power meter, were delivered to the protein solder surface, resulting in irradiances of 4.9, 6.6, 8.2, and 12.3 W/cm$^2$, respectively. A translation stage moved the tissue under the beam at a scanning speed of 0.3 m/s. Eight continuous passes were used for the liquid protein solder, and four continuous passes were used for the solid protein solder. The length of each pass was 3.0 mm; thus, the total exposure times for the liquid protein solder and solid protein solder were 40 seconds and 80 seconds, respectively.

### Tissue Preparation

Bovine thoracic aortas were obtained from a slaughter house (Taylor Meat Company, Taylor, TX). The aortas were rinsed with phosphate-buffered saline (PBS), wrapped in saline-soaked gauze, and stored at −70°C [22] until required. Before use, aortas were thawed and then cut into rectangular specimens having approximate dimensions of 2 × 1 cm. The excess adventitia and media were trimmed to obtain a specimen thickness of approximately 1 mm.

### Surface Procedure

A full-thickness incision was cut through the specimen width by using a scalpel and opposing ends were placed together. Laser soldering was performed on the intima of the aorta.

By using the premixed dye-solder technique, ICG dye, with a concentration of 0.25 mg/ml, was added to the protein solders to enhance light absorption. By using the technique described in Part I, the dyed solder was placed perpendicularly across the junction of the severed aorta specimen and denatured with a continuous pass of the diode laser output.

By using the separate dye-solder technique, a separate ICG solution (concentration of 10 mg/ml) was prepared with deionized water. A precision micropipette (Hamilton Company) was used to smear a 0.5-μl drop of the ICG solution across the tissue surface to be treated. The undyed liquid or solid solder was then placed perpendicularly across the junction of the severed aorta specimen, directly on top of the ICG solution, and denatured with a continuous pass of the diode laser output.

The exposure time required for the liquid protein solder to coagulate was about twice the optimal time required for the solid protein solder repairs. As mentioned in Part I of this study, the longer exposure times required were most likely attributable to two effects: (1) the latent heat required during vaporatization of water in the solder, and (2) the higher thermal conductivity of the liquid solder causing the heat to spread out through the solder (unpublished data). The investigation was divided into two parts during which a total of 1,440 bovine aorta tissue repairs were performed in vitro and tested.

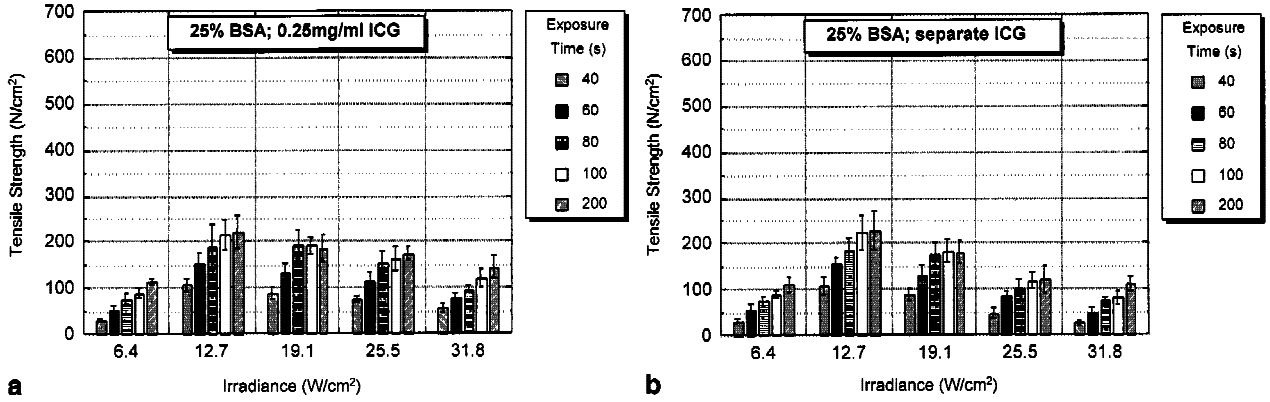


Fig. 1. Tensile strength of repairs formed with liquid protein solder (25% BSA) by using (**a**) the premixed dye-solder technique, and (**b**) the separate dye-solder technique. Each graph shows results from the five different irradiation times given in the legends. Each bar shows the mean and standard deviation for 10 repairs. BSA, bovine serum albumin; ICG, indocyanine green.

### Tensile Strength Analysis

Tensile strength measurements were performed to test the integrity of the resultant repairs immediately after the laser procedure by using the gravity-based tensiometer described in Part I of this study [22,23]. Ten repairs were performed and tested for the four variations of protein solder at each of the five laser irradiances and exposure times investigated. Thus, a total of 1,000 tissue repairs were performed and tested for this analysis.

### Hydration Study

The effect of hydration on bond stability was studied by using both tensile strength analysis and scanning electron microscopy. Immediately after the laser procedure, specimens requiring tensile strength analysis were soaked in PBS for a designated hydration period (1 hour, 1 day, 2 days, 1 week) then tested to determine the tensile strength of the repair. Acute tensile strength measurements were also made on a control group. Five repairs were performed and tested for the four variations of protein solder at each of the four laser irradiances and five hydration periods (including control) investigated. Thus, a total of 400 tissue repairs were performed in this study.

The remaining specimens were prepared for SEM analysis. Specimens were re-cut, with a scalpel, along the line of the original tissue cut, to expose the solder/tissue cross-section. Half of each specimen was fixed immediately in 2.5% glutaraldehyde as a control and the other half was soaked in PBS for the designated hydration period. After each hydration period, all specimens were removed from the PBS, fixed in glutaraldehyde, and prepared for SEM analysis. SEM analysis was performed on 40 specimens using a JEOL 840 scanning electron microscope.

## RESULTS

### Tensile Strength Analysis

Results of tensile strength measurements made on successfully repaired tissue specimens as a function of irradiance and exposure time are presented in Figures 1 and 2. The results of experiments conducted with liquid protein solder (25% BSA) by using the premixed dye-solder (0.25 mg/ml ICG) and the separate dye-solder techniques are presented in Figure 1a and b, respectively, and the results of experiments conducted with solid protein solder (60% BSA) by using the two techniques are presented in Figure 2a and b, respectively. The tensile strength for each value of laser irradiance and exposure time was determined from the mean values for 10 repairs. The standards deviation is also shown in each case. Repairs that failed to sustain a tensile strength above 10 N/cm$^2$ were deemed to be unsuccessful and are not presented in the results of Figures 1 and 2. By using the premixed dye-solder technique, 10 of the 260 liquid protein solder repairs and 1 of the 251 solid protein solder repairs tested fell into the unsuccessful category. By using the separate dye-solder technique, 8 of the 258 liquid protein solder repairs and 0 of the 250 solid protein solder repairs tested were unsuccessful. This represented dehiscence rates of 3.9%, 0.4%, 3.1%, and 0%, respectively.

As discussed in Part I of this study [23], the liquid and solid protein solders exhibited different

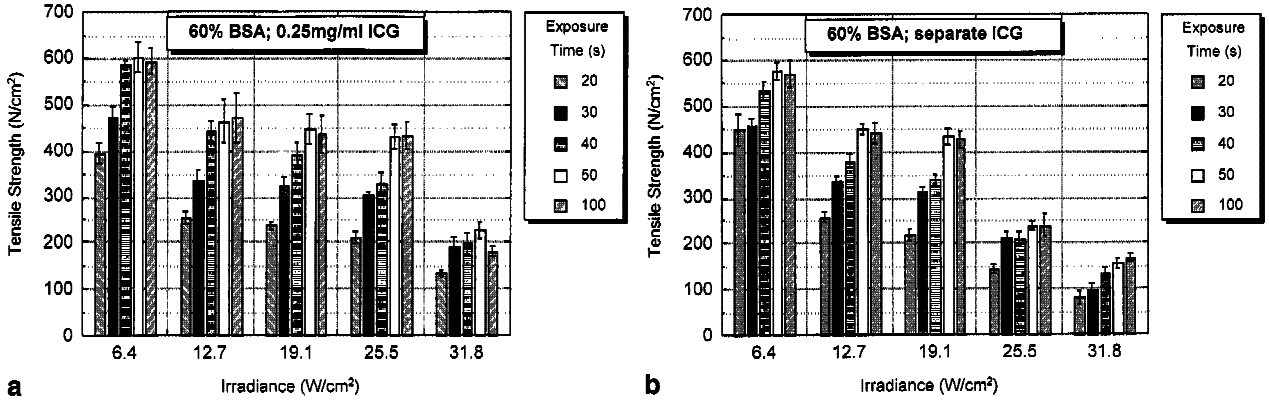


Fig. 2. Tensile strength of repairs formed with solid protein solder (60% BSA) by using (**a**) the premixed dye-solder technique, and (**b**) the separate dye-solder technique. Each graph shows results from the five different irradiation times given in the legends. Each bar shows the mean and standard deviation for 10 repairs. BSA, bovine serum albumin; ICG, indocyanine green.

mechanisms of failure. The liquid protein solder broke into two halves, but each remained attached to the tissue. The solid protein solder, however, remained intact but detached from the tissue. Nevertheless, the results in Figures 1 and 2 are presented in terms of the cross-section tensile strength for both the liquid and the solid solder. As shown in the figures, the overall pull-apart strength of repairs formed using the solid protein solder was significantly higher than the strength of the liquid protein solder repairs.

The conclusions made in Part I of this study are reinforced by the data in Figures 1 and 2: (1) for the parameters investigated, tensile strength of the resulting repairs decreased significantly with increasing irradiance except for the 6.4 W/cm$^2$ irradiance of the liquid solders, (2) an exposure time existed after which further irradiation of the solid protein solder (60% BSA) did not improve the tensile strength of the repair, (3) increasing BSA concentration from 25 to 60% greatly improved the tensile strength of the repair.

By using the premixed dye-solder technique with the liquid protein solder, tensile strength increased from a maximum of 114 ± 6 N/cm$^2$ to a maximum of 220 ± 35 N/cm$^2$ when irradiance doubled from 6.4 W/cm$^2$ to 12.7 W/cm$^2$ (Fig. 1a). However, at this point, tensile strength fell to a low of 144 ± 23 N/cm$^2$ as irradiance increased to 31.8 W/cm$^2$. By using the solid protein solder, tensile strength decreased by approximately 62% from a maximum of 602 ± 32 N/cm$^2$ to a maximum of 226 ± 17 N/cm$^2$ when irradiance was increased from 6.4 W/cm$^2$ to 31.8 W/cm$^2$ (Fig. 2a).

By using the separate dye-solder technique with the liquid protein solder, tensile strength increased from a maximum of 110 ± 15 N/cm$^2$ to a maximum of 228 ± 41 N/cm$^2$ when irradiance doubled from 6.4 W/cm$^2$ to 12.7 W/cm$^2$ (Fig. 1b). Beyond this point, tensile strength fell to a low of 109 ± 16 N/cm$^2$ as irradiance increased to 31.8 W/cm$^2$. By using the solid protein solder, tensile strength decreased by approximately 73% from a maximum of 578 ± 20 N/cm$^2$ to a maximum of 166 ± 8 N/cm$^2$ when irradiance was increased from 6.4 W/cm$^2$ to 31.8 W/cm$^2$ (Fig. 2b).

In Part I of this study, the tensile strength of native aorta (~1 mm thick) was found to be 596 ± 31 N/cm$^2$ [23]. The maximum tensile strengths achieved by using the premixed and separate dye-solder techniques with solid solder were comparable to this (602 ± 32 N/cm$^2$ and 578 ± 20 N/cm$^2$, respectively). However, the maximum tensile strengths achieved for the liquid solder repairs (220 ± 35 N/cm$^2$ and 228 ± 41 N/cm$^2$, respectively) were significantly less than that of native aorta and the solid solder repairs.

As illustrated in Figure 2a and b, there was an optimal exposure time for the solid protein solder for which further irradiation did not improve the tensile strength of the repairs. An optimal exposure time was not always observed for the liquid solder, however, which required much longer times to achieve coagulation (see Fig. 1a,b). As mentioned in Part I, this finding was most likely caused by the latent heat required during vaporization of water in the solder [23]. Investigation of longer periods of irradiation should determine with more certainty an optimal exposure time for the liquid protein solder.

Irradiance and exposure time for optimal strength by using both the premixed dye-solder and the separate dye-solder techniques were de-

**TABLE 1. Summary of Optimised Results From Tensile Strength Analysis***

| Solder | Maximum tensile strength ($N/cm^2$) | Laser irradiance ($W/cm^2$) | Exposure time (s) |
|---|---|---|---|
| 25% BSA; 0.25 mg/ml ICG | 220 ± 35 | 12.7 | 100 |
| 25% BSA; separate ICG | 228 ± 41 | 12.7 | 100 |
| 60% BSA; 0.25 mg/ml ICG | 602 ± 32 | 6.4 | 50 |
| 60% BSA; separate ICG | 578 ± 20 | 6.4 | 50 |

*The tensile strength of native aorta was found to be 596 ± 31 $N/cm^2$. BSA, bovine serum albumin; ICG, indocyanine green.

termined from these results to be 12.7 $W/cm^2$ for 100 seconds with the liquid protein solder repairs and 6.4 $W/cm^2$ for 50 seconds with the solid protein solder repairs. (Note that the investigation in the Hydration Study section below confirms that 6.4 $W/cm^2$, the minimum irradiance investigated in this section, was the optimal irradiance for solid protein solder repairs.) Table 1 summarizes the results of the tensile strength analysis performed in this investigation.

### Hydration Study

**Tensile strength measurements.** Results of tensile strength measurements made on laser-solder repaired specimens after various periods of hydration as a function of irradiance are presented in Figures 3 and 4. The results of experiments conducted with the liquid protein solder (25% BSA) by using the premixed dye-solder technique (0.25 mg/ml ICG) and the separate dye-solder technique are presented in Figure 3a and b, respectively. The results of experiments conducted with the solid protein solder (60% BSA) by using the premixed dye-solder technique (0.25 mg/ml ICG) and the separate dye-solder technique are presented in Figure 4a and b, respectively. The tensile strengths for each value of laser irradiance and each hydration period were determined from the mean values for five repairs. The standard deviation is also shown.

Repairs formed by using the separate dye-solder technique were more stable during hydration than were repairs formed by using the premixed dye-solder technique (0.25 mg/ml ICG). The tensile strength of the liquid solder repairs formed by using the separate dye-solder technique (Fig. 3b) reduced by only 5 to 10% with further hydration up to 1 week in contrast to the 20 to 25% reduction experienced by repairs formed by using the premixed dye-solder technique (Fig. 4a). Likewise, the reduction in tensile strength of solid solder repairs formed by using the premixed dye-solder technique of approximately 5% with further hydration up to 1 week was not observed when the separate dye-solder technique was used (reduction ~0%) (Fig. 4b). Independent of the technique used, however, the reduction in tensile strength of repairs formed by using the solid protein solder was not significant (Student's $t$-test: $p < 0.05$).

**Scanning electron microscopy analysis.** Scanning electron micrographs of six of the specimens treated by using the separate dye-solder technique are shown in Figure 5. A good coagulum is seen at the solder/tissue interface for both the liquid and the solid protein solders. Repairs formed by using the separate dye-solder technique with the liquid protein solder remained well adhered to the tissue substrate even after 1 week of hydration (Fig. 5e). This finding is in contrast to the results of Part I of this study, for which SEM analyses showed repairs formed by using the premixed dye-solder technique with liquid protein solder (0.25 mg/ml ICG) to suffer solder detachment after only 1 hour of hydration [23]. Thus, the separate dye-solder technique may produce a more reliable fusion between the solder and tissue substrate than the premixed dye-solder technique. However, as observed in the unpublished results of a histologic study comparing the two techniques, there is a trade-off between the extent of thermal damage caused to the underlying tissue and the stability of the formed bond.

## DISCUSSION

Augmenting laser-tissue repair techniques with protein solder significantly improves the postoperative results. However, low initial tensile strength of the resultant repairs and the further reduction of tensile strength with rehydration of the repairs (refer to Part I) [14,22,23] can be a

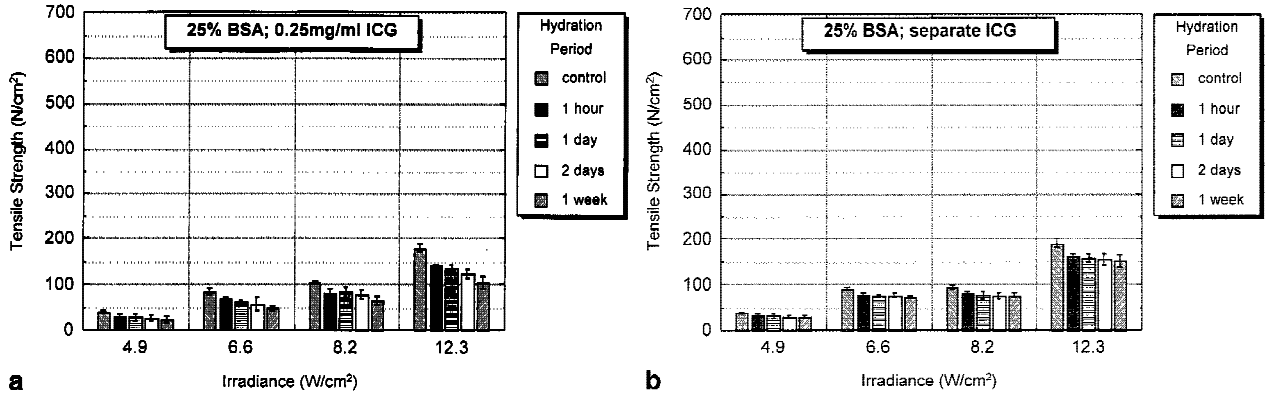


Fig. 3. Tensile strength of repairs formed with liquid protein solder (25% BSA) by using (**a**) the premixed dye-solder technique (0.25 mg/ml ICG), and (**b**) the separate dye-solder technique (dye solution concentration of 10 mg/ml ICG). Each solder specimen was irradiated for 80 seconds with a scanning laser beam. Results are shown for the control and after soaking in phosphate-buffered saline for designated hydration periods of 1 hour, 1 day, 2 days, or 1 week. Each bar shows the mean and standard deviation for five repairs. BSA, bovine serum albumin; ICG, indocyanine green.

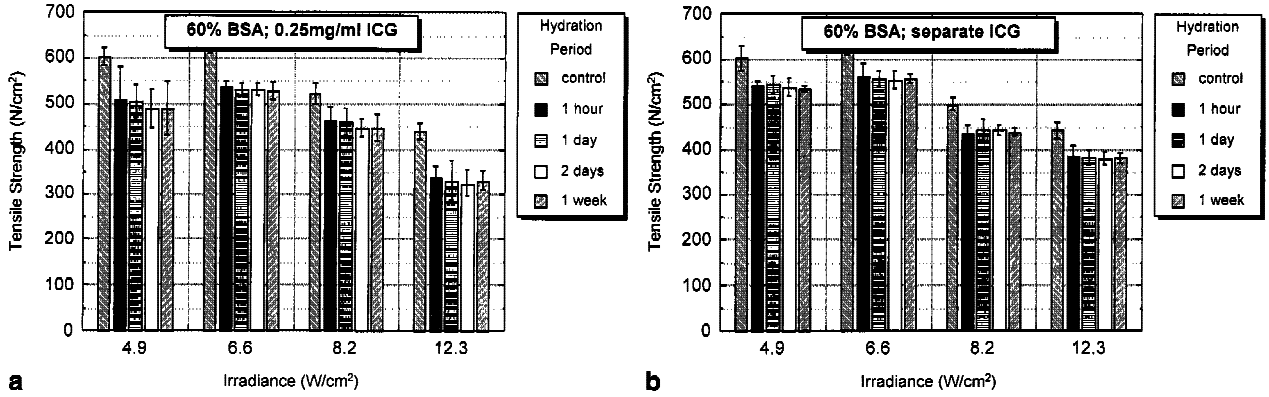


Fig. 4. Tensile strength of repairs formed with solid protein solder (60% BSA) by using (**a**) the premixed dye-solder technique (0.25 mg/ml ICG), and (**b**) the separate dye-solder technique (dye solution concentration of 10 mg/ml ICG). Each solder specimen was irradiated for 40 seconds with a scanning laser beam. Results are shown for the control and after soaking in phosphate-buffered saline for designated hydration periods of 1 hour, 1 day, 2 days, or 1 week. Each bar shows the mean and standard deviation for five repairs. BSA, bovine serum albumin; ICG, indocyanine green.

significant drawback to the technique, particularly when using liquid protein solders.

One cause of the low initial tensile strength of repairs is thought to be the premature denaturation of the surface of the protein solder creating a thermal barrier to the rest of the solder, in particular, the face of the solder in contact with the tissue. Laser light around 800 nm is poorly absorbed by either water or tissues; therefore, it is primarily absorbed by the ICG dye contained within the protein solder. Thus, the heat source with ICG-doped protein solders is a maximum at the anterior surface of the solder and decreases exponentially (Beer's Law). A temperature gradient is established over the depth of the solder. This finding is particularly evident in the scanning electron micrographs of Part I of this study showing repairs formed with liquid protein solder [23]. Depending on the temperature gradient and the laser irradiance and exposure time used, the upper portion of the solder tends to coagulate while the most critical region, i.e., the solder/tissue interface, does not coagulate fully. The use of higher irradiances does not improve the results, because this use tends to lead to air vacuole and char formation at the top of the solder, presenting heat conduction to the lower layer of the solder. Figure 6 shows an example from a preliminary study for which a high irradiance of 25.5 W/cm$^2$ was used to denature liquid protein solder. Under these circumstances, further irradiation does not improve the tensile strength of the re-

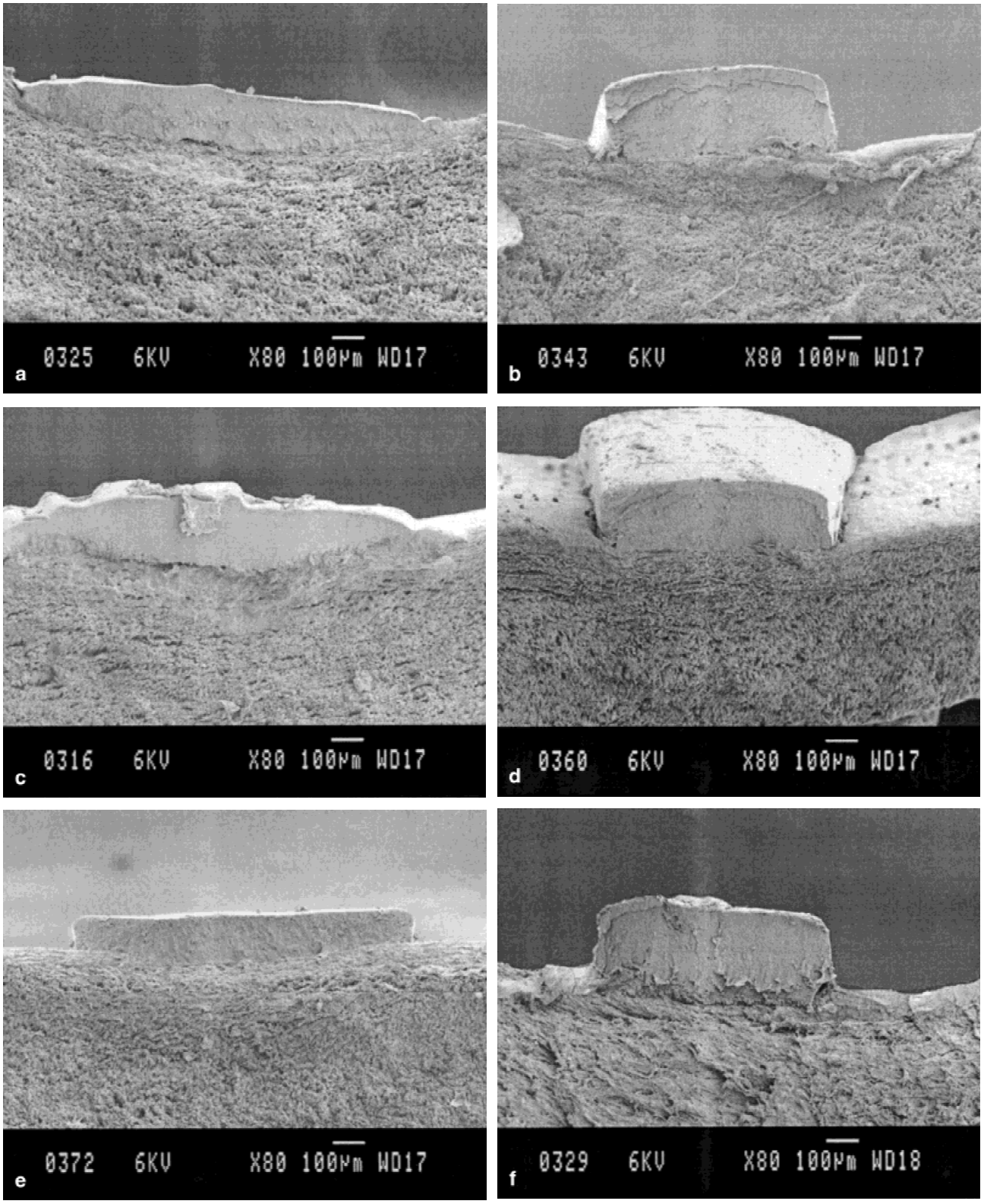


Fig. 5. Cross-section of tissue repairs formed by using the separate dye-solder technique (dye solution concentration of 10 mg/ml ICG) after various periods of hydration: (**a**) liquid protein solder (25% BSA) control; (**b**) solid protein solder (60% BSA) control; (**c**) liquid protein solder after 1 hour of hydration; (**d**) solid protein solder after 1 hour of hydration; (**e**) liquid protein solder after 1 week of hydration; and (**f**) solid protein solder after 1 week of hydration. Liquid solders were denatured by using the optimised irradiance of 12.3 W/cm$^2$ determined in this study. Solid solders were denatured by using the optimised laser irradiance of 6.6 W/cm$^2$. In a, the liquid nature of the solder meant that control of its thickness was virtually impossible. Evidence of thermal coagulation can be seen in the underlying tissue. In c, evidence of thermal coagulation can be seen clearly in the underlying tissue. In e, the solder appears to be well adhered to the tissue, despite the long period of hydration.

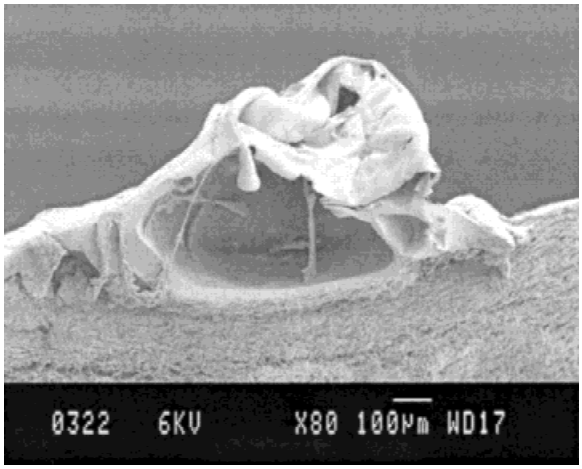


Fig. 6. Scanning electron micrograph of repair formed by using liquid protein solder containing 0.25 mg/ml ICG. Solder was denatured by using an irradiance of 25.5 W/cm$^2$ for 100 seconds. Large air vacuoles are seen in the solder. Heat conduction to the lower layers of the solder was prevented, resulting in inadequate coagulation of the solder at the vital solder-tissue interface and the corresponding low tensile strength of repairs formed using such high irradiances.

pair. If the lower layer of the solder is not denatured, it cannot fuse properly with the tissue and will tend to dissolve quickly, allowing the tissue to separate from the solder. This outcome is a particular concern when the repair is submerged in a hydrated environment.

The strength of the solder-assisted tissue repair is, thus, dependent not only on the concentration of protein in the solder, but also on appropriate temperatures being reached at the solder/tissue interface. Fusion between the solder and tissue (without excessive thermal, damage to the surrounding tissue) is obtained when a temperature allowing molecular mobility is reached, which permits intermingling of the protein helices and polymerization to occur.

To address the concern of premature denaturation at the solder surface, an alternative soldering technique was investigated to determine whether it would result in more stable solder-tissue coagulation. Instead of mixing the absorbing dye with the solder, a thin layer of ICG dye was smeared over the surface to be treated and the pure albumin protein solder was placed directly on top of the dye. Because the laser light was primarily absorbed by the ICG dye, this thin layer of ICG solution formed the heat source to the solder and the aorta intima.

The tissue was stained a dark green color when the ICG dye was applied directly to its surface. In contrast, applying the ICG-doped protein solder to the tissue surface caused the tissue to turn a very pale green color. These observations were consistent with a previous study by La Joie et al., in which it was shown that ICG in water penetrated the intimal layer of porcine aorta to depths varying between 100 μm and 400 μm, with an average of 200 μm, independent of time soaked [24]. When mixed with the solder, ICG penetration in the tissue was reduced (<100 μm) and nonuniform. These differences are thought to be because the ICG dye was already bonded to the protein in the solder mixtures. In the present study, the initial ICG concentration of 10 mg/ml used for the separate dye-solder technique was, thus, reduced because of penetration of the dye into the solder and tissue.

Tensile strength measurements of repairs formed by using the separate dye-solder technique and irradiances ≤19.1 W/cm$^2$ (Figs. 1b, 2b), were not significantly different to those found using the premixed dye-solder technique when solder containing 0.25 mg/ml ICG was used (Figs. 1a, 2a). In Part I with premixed solder, repairs with solders containing 0.25 mg/ml ICG were found to be on average 20% stronger than those with 2.5 mg/ml ICG solder, the ICG dye concentration commonly used by other researchers. The reduced ICG dye concentration of the solder increased the penetration depth of the laser light from approximately 35 μm to 85 μm (cf. solder thickness ~150 μm), resulting in a more even temperature gradient across the solder. This simple change appeared to be sufficient to produce a more even temperature gradient across the solder, making the potential tensile strength benefits of separate dye-solders irrelevant. Beyond an irradiance of 19.1 W/cm$^2$, thermal damage to tissue in repairs formed by using the separate dye-solder technique (unpublished data from histologic analysis) may have led to decreased tensile strength of the resultant repairs in comparison to repairs formed by using the premixed dye-solder technique.

Superior results in terms of tensile strength upon hydration were achieved by using the separate dye-solder technique with the liquid protein solder (Fig. 3b). The effect was not as dramatic with the solid protein solder (Fig. 4b). Scanning electron microscopy analysis also showed the separate dye-solder technique to produce repairs that were more stable during hydration. This finding was particularly evident for the liquid protein solder repairs, which remained well adhered to the tissue substrate even after 1 week of hydration. Repairs formed by using the premixed

dye-solder technique were shown to suffer solder detachment after 1 hour of hydration in Part I of this study [23]. These results support the findings of Chan, in which a liquid protein solder (25% BSA, 2.5 mg/ml ICG, 0.5% sodium hyaluronate) was used to repair bovine aorta specimens in vitro [22]. No statistical difference was observed in the ultimate tensile strength of repairs formed by using both techniques; however, scanning electron microscopy analysis suggested that the ICG layer concentrated at the solder-tissue interface provided a more reliable solder-tissue fusion than coagulating the solder with premixed ICG.

Irradiation through the solder, causes direct irradiation of the rear surface of the dye stain layer, within the solder and away from the tissue. Thermal diffusion then brings the heat to the repair site. Another alternative technique achieving the same aim is to irradiate through the tissue to denature the ICG-doped protein solder. This technique allows direct irradiation of the solder/tissue interface. The two alternative techniques are seen to differ when the absorption by the stain layer is sufficiently high that little laser energy directly reaches the solder tissue surface. In a study by Jacques et al., it was postulated (but not examined) that the later approach should be better [25].

For the separate dye-solder technique to be a substitute for the premixed solder technique, it must either offer benefits of speed of performance or be shown to give more promising results in terms of recovery. Applying the premixed dye-solder to the substrate junction was far simpler and less time consuming than applying the dye and solder separately. The low-viscosity dye solution, used with the separate dye-solder technique, tended to flow all over the immediate tissue surface, increasing the risk of unnecessary heating of healthy tissue. The addition of hyaluronate to the dye solution might solve the viscosity problem [6]. However, the separate dye-solder technique does produce repairs that are more stable during hydration, and the shelf-life of the solder is increased because the dye can be easily mixed as required, thus conserving its spectral absorbency properties.

## CONCLUSIONS

Two laser-assisted tissue soldering techniques have been evaluated for repairing aorta incisions in vitro. One technique used a premixed albumin/ICG dye solder and the other used a separate albumin solder and ICG dye. No significant difference was found in the tensile strength of the resulting welds. In both cases, an optimal irradiance and irradiation time were found to exist and tensile strength was improved by increasing albumin concentration. However, the separate dye-solder technique offers the benefit of longer storage times of the protein solder.

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